

Cost of Deconstruction Depots for Diversified, Waste-Based Lignocellulosic Sugars Using Distillable Solvents

Background/Objective

- Evaluate cost-effectiveness of sugar production from 22 biomass types via butylamine-based deconstruction and identify process bottlenecks and improvement opportunities.

Approach

- Develop field-to-sugar process models for 22 biomass feedstocks.
- Perform mass and energy balances followed by economic analyses across different scenarios.

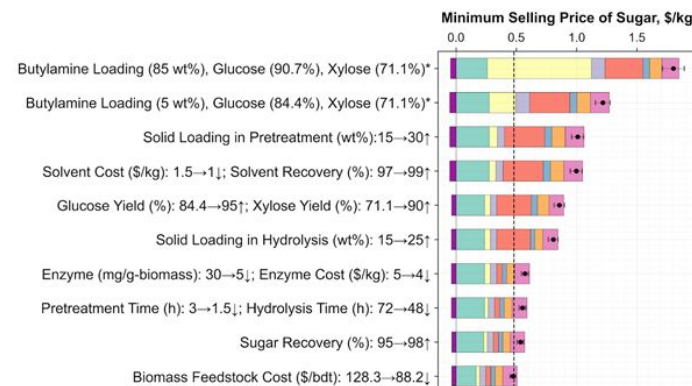
Results

- Butylamine enables feedstock-flexible deconstruction but needs higher sugar yields and lower enzyme use for viability.
- Meeting targets (Fig. 1A) lowers sugar price to <\$0.50/kg (Fig. 1B).

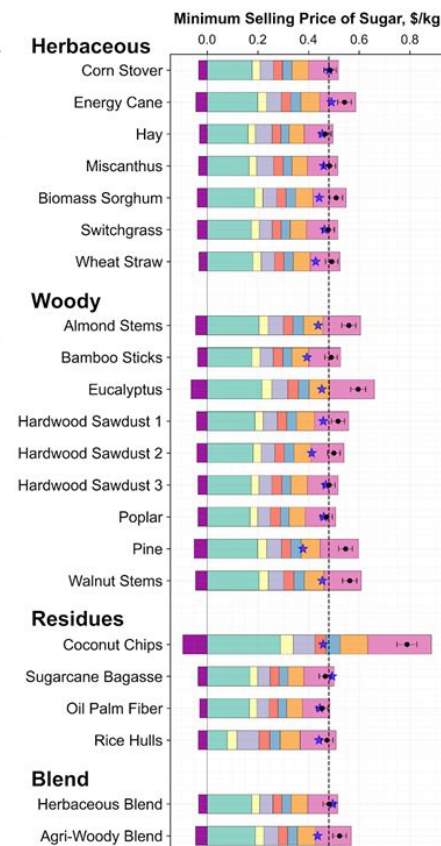
Significance/Impacts

- Demonstrates that butylamine-based biomass deconstruction can enable economically viable, feedstock-flexible biorefineries by meeting defined performance thresholds.

A. Stepwise Reduction Toward Optimal Future Sugar Price



B. Optimal Future Sugar Selling Price



C. Price Point Where High-Lignin Outperforms Low-Lignin

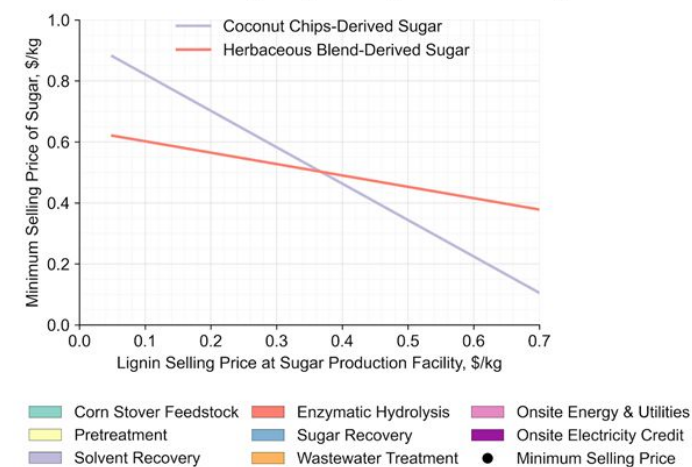


Figure caption: Minimum sugar selling price: (A) Stepwise price reduction using poplar as a representative feedstock; (B) optimal prices across diverse biomass feedstocks; and (C) cut-off lignin price where coconut outperforms the herbaceous blend. Vertical dashed lines (----) indicate the average reported selling price of corn stover-derived sugar (\$0.48/kg) produced via dilute sulfuric acid pretreatment. *Experimental data.

Uncovering Sequence and Structural Characteristics of Fungal Expansin-Related Proteins With Potential to Drive Substrate Targeting

Background/Objective

- Expansins are proteins that bind to and disrupt plant and fungal cell walls for cell growth and cell wall modification/deconstruction
- The goal was to characterize the structural characteristics of fungal expansins for substrate targeting and modification

Approach

- Structural prediction, comparative analysis, and *in vitro* studies with recombinant proteins were performed to evaluate substrate binding interactions

Results

- Fungal expansin-related proteins had distinct substrate binding preferences, highlighting functional diversity
- Correlations identified between specific protein sequences and their predicted structural features involved in substrate binding

Significance/Impacts

- Understanding mechanisms of fungal expansin-related proteins will advance applications in biomass deconstruction
- Results provide a framework for future engineering of proteins with tailored substrate targeting capabilities

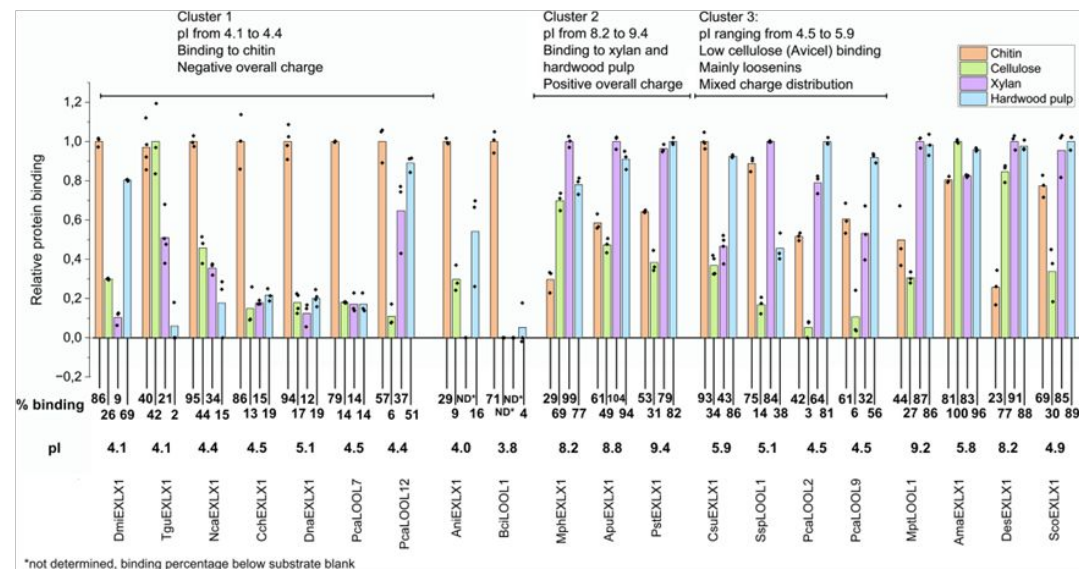


Figure caption: Binding by recombinant fungal expansin-related proteins to selected substrates at pH 5.0; chitin (orange), microcrystalline cellulose (Avicel, green), oatspelt xylan (purple), hardwood kraft pulp (blue).

High-quality draft genome sequence of *Thermobifida halotolerans* DSM 44931

Background/Objective

- *T. halotolerans* is a filamentous actinomycete that can degrade different types of biomass and polymers
- Objective: Improve the genome for this bacterial species

Approach

- Harvested DNA was sequenced using PacBio sequencing technology
- The genome draft assembly was annotated using the JGI Integrated Microbial Genomes annotation pipeline v5.1.9

Results

- A high-quality draft genome of *T. halotolerans* was created and annotated to provide metabolic insight into this species

Significance/Impacts

- This genome improves our understanding of *T. halotolerans* biology and expands our ability to use actinomycetes for biotechnological applications

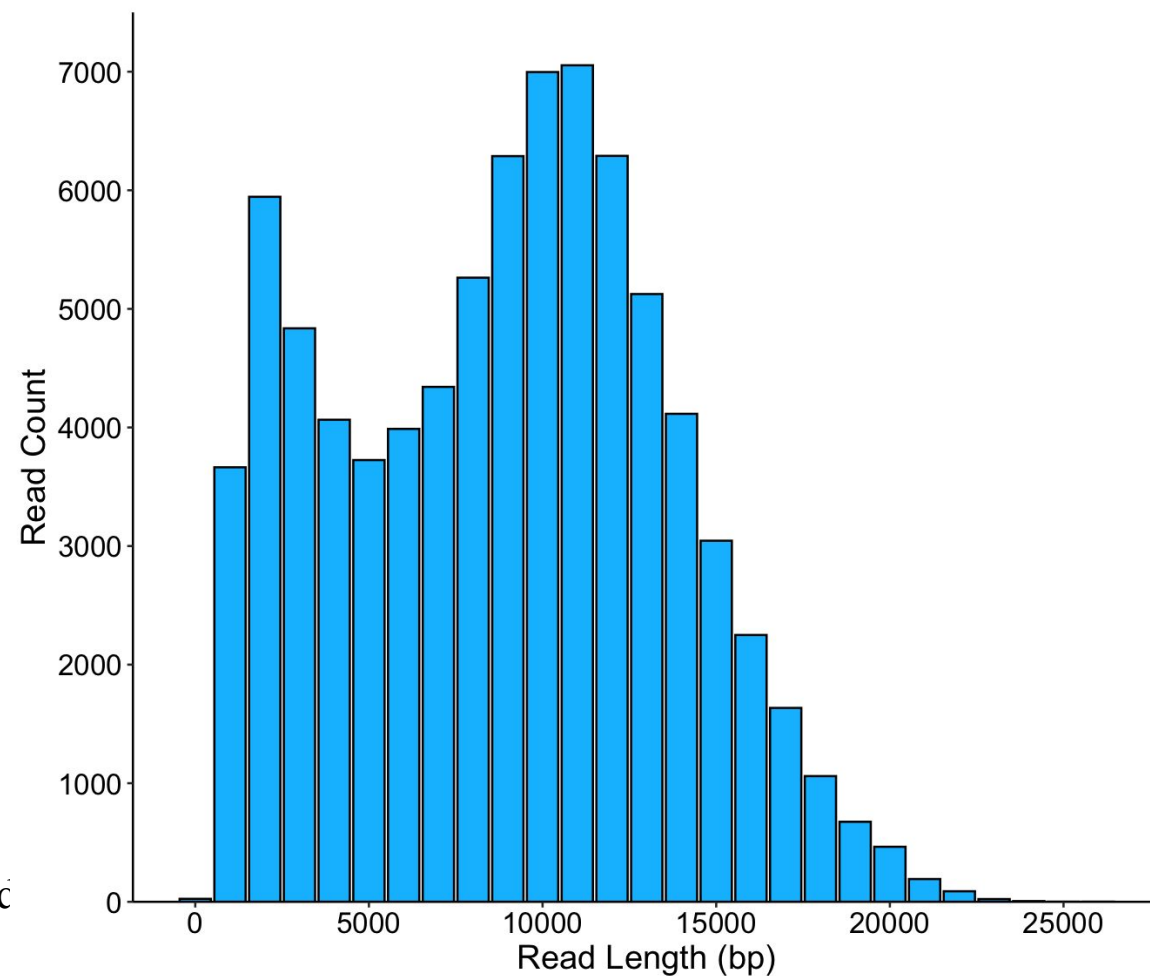


Figure caption: A histogram of the length frequencies of the CCS reads generated by the PacBio Sequel platform.

Distillable Amine-Based Solvents for Effective Pretreatment of Multiple Biomass Feedstocks

Background/Objective

- Conventional biomass pretreatments often require high solvent use, washing steps, and face recovery challenges.
- Evaluate distillable amine-based solvent pretreatment across diverse lignocellulosic feedstocks.

Approach

- Screened 5 solvents and 22 feedstocks for solvent removal and enzymatic digestibility.
- Scaled up butylamine pretreatment and tested hydrolysate microbial growth.

Results

- Butylamine pretreatment achieved >90% glucose release and >99% solvent removal for agricultural mixed residues.
- Scale-up improved yields; hydrolysates supported microbial growth with strain-specific tolerance.

Significance/Impacts

- Demonstrates a feedstock-agnostic, distillable pretreatment pathway that simplifies operations by avoiding washing.
- Provides a scalable, economically viable pretreatment compatible with industrial microbes, advancing bio-based fuels and chemicals.

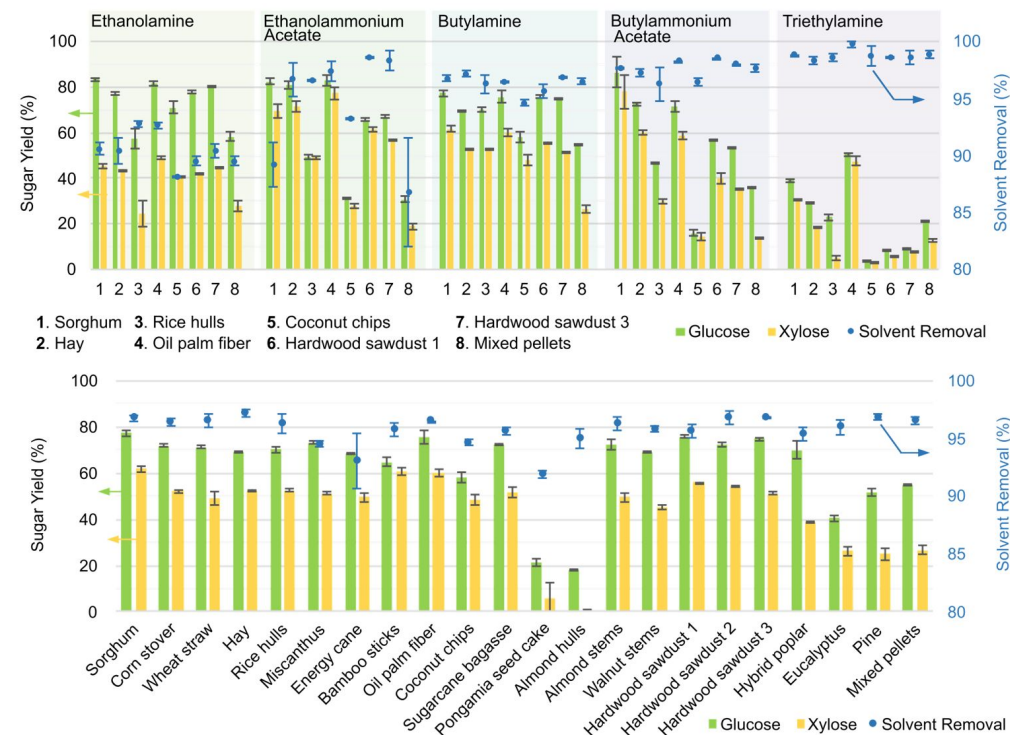
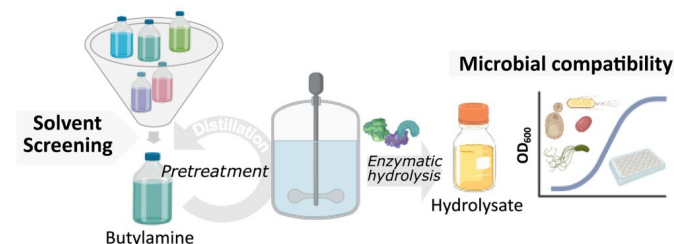


Figure caption: Overview (top); Pretreatment of diverse feedstocks with different solvents followed by enzymatic hydrolysis (middle); Butylamine pretreatment enabling solvent removal and sugar release (xylose and glucose) across biomass feedstocks (bottom).

FluxRETAP: A REaction TArget Prioritization Genome-Scale Modeling Technique for Selecting Genetic Targets

Background

- **Metabolic engineering is rapidly evolving** as a result of new advances in synthetic biology, as well as the development of machine learning tools (ML) for biology.
- However, **selecting genetic engineering targets** that effectively guide the metabolic engineering process is **still challenging**.

Approach

- FluxRETAP, a simple and computationally inexpensive method that leverages genome-scale models (GSMs) for **suggesting targets for genetic overexpression, downregulation or deletion** to increase production.

Results

- FluxRETAP captured **100% of reaction targets** experimentally verified to improve *Escherichia coli* isoprenol production, **50% of targets** that experimentally improved taxadiene production in *E. coli* and **~60% of genetic targets** from a verified minimal constrained cut-set in *Pseudomonas putida*, while providing additional high priority targets that could be tested.

Significance

- Overall, FluxRETAP is an **efficient algorithm** for identifying a prioritized list of testable **genetic and reaction targets** to improve production of desired metabolites

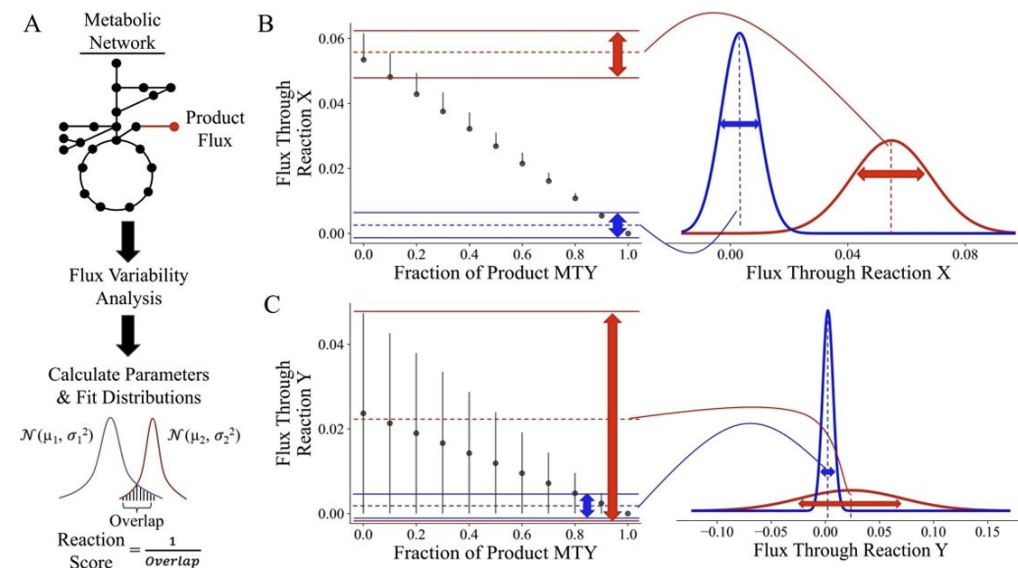


Figure caption: FluxRETAP performs Flux Variability Analysis (FVA) at several fractions of the maximum theoretical yield (MTY) of production (e.g., at 10, 20, 30 ... 100% of the maximum flux towards the final product), finding for each reaction the range of fluxes compatible with each fraction. FluxRETAP then calculates the overlap between initial (first two fractions, e.g., 10, 20%) and final (last two fractions, e.g., 90, 100%) flux distributions by fitting gaussian distributions to these flux ranges and finding their overlap. FluxRETAP uses as score the inverse of the overlap, because we are interested in the reactions for which there is the largest change between low production and high production (i.e. smallest overlap between initial and final flux distributions).

Life-Cycle Emissions and Human Health Implications of Multi-Input, Multi-Output Biorefineries

Background/Objective

- There are economic and environmental advantages associated with integrating AD & cellulosic fuels in a single facility.

Approach

- Conduct detailed life-cycle assessment across 7 different scenarios using corn stover, DMR pretreatment, and co-processing of multiple feedstocks

Results

- Potential for large human health advantages from reduced ammonia emissions, all multi-IO scenarios outperform baseline

Significance/Impacts

- Indicates that future biorefineries can diversify their feedstocks to achieve economic and environmental benefits, including air quality, eutrophication, and acidification

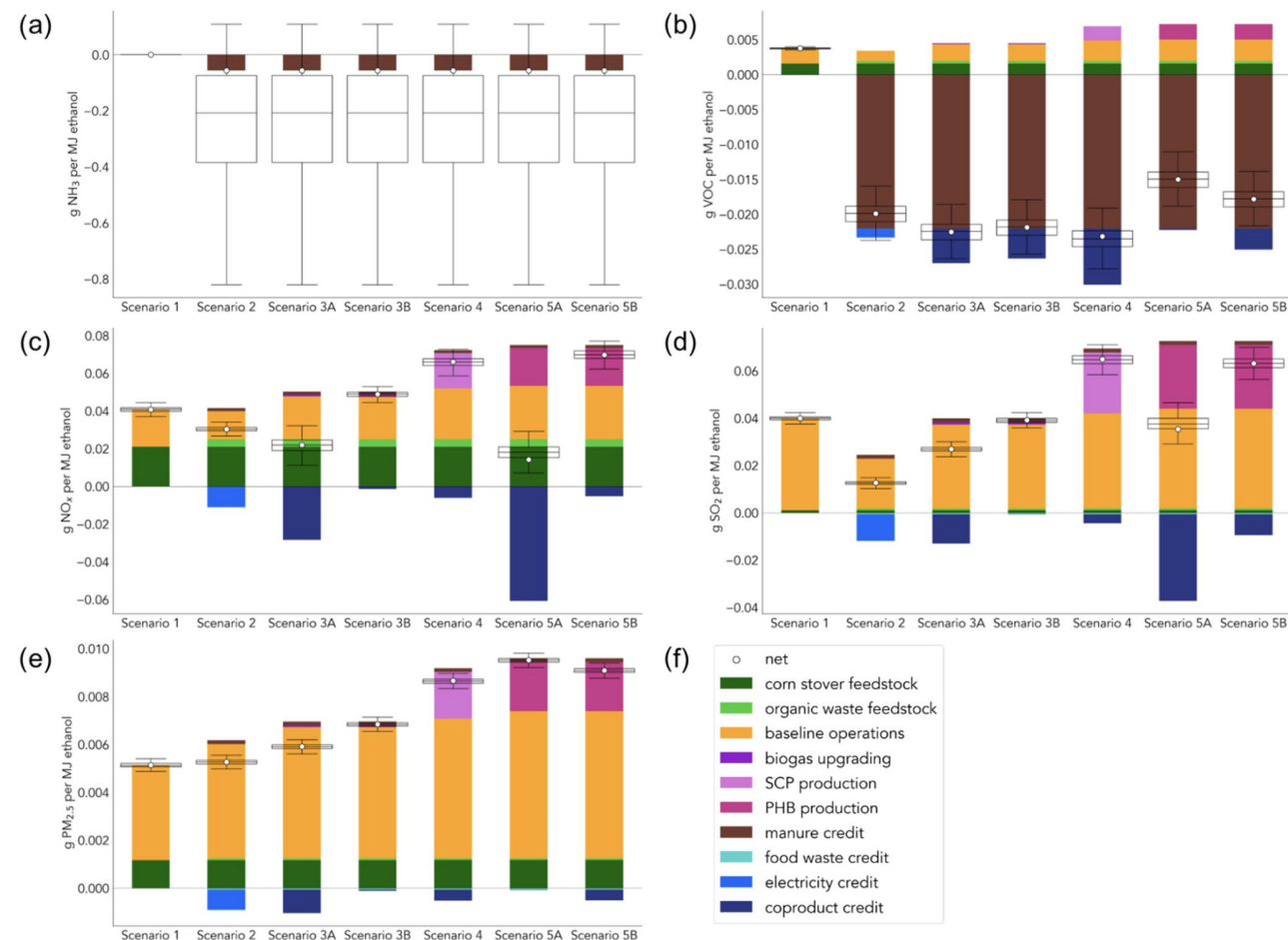


Figure 4. Net effects of each scenario on system-wide life-cycle air pollution emissions. The distribution of results from Monte Carlo simulations is shown by the box and whisker plots, where the boxes extend from the first to the third quartile of the data with a line at the median. The whiskers extend from the boxes to the farthest value that is within 1.5× the interquartile range of the boxes. (a) Life-cycle NH₃ emissions. (b) Life-cycle VOC emissions. (c) Life-cycle NO_x emissions. (d) Life-cycle SO₂ emissions. (e) Life-cycle PM_{2.5} emissions. (f) This legend applies to all plots (a–e) in this figure.

Nordahl, S. L., et al. Environmental Science & Technology. doi: 10.1021/acs.est.4c12920 (JBEI #1260)

The anaerobic fungus *Neocallimastix californiae* shifts metabolism and produces melanin in response to lignin-derived aromatic compounds

Background/Objective

- We previously reported that anaerobic gut fungi (AGF) deconstruct lignin.
- The relationship between AGF and lignin deconstruction products, aromatics derived from lignocellulose, has remained undefined.

Approach

- We introduced lignin, extracted from lignocellulose, to cultures of AGF.
- We measured the metabolomic and transcriptomic responses of AGF to extracted lignin additions and compared those to controls with no lignin.

Results

- Additions of lignin induced differences in AGF metabolomics, transcriptomics, and macroscopic morphology.
- Additions of lignin caused AGF to produce melanin.

Significance/Impacts

- The findings indicate AGF can transform aromatics anaerobically, opening a new avenue of investigation for biological upgrading of lignocellulose.
- Melanin produced by AGF has evolutionary and biochemical implications.

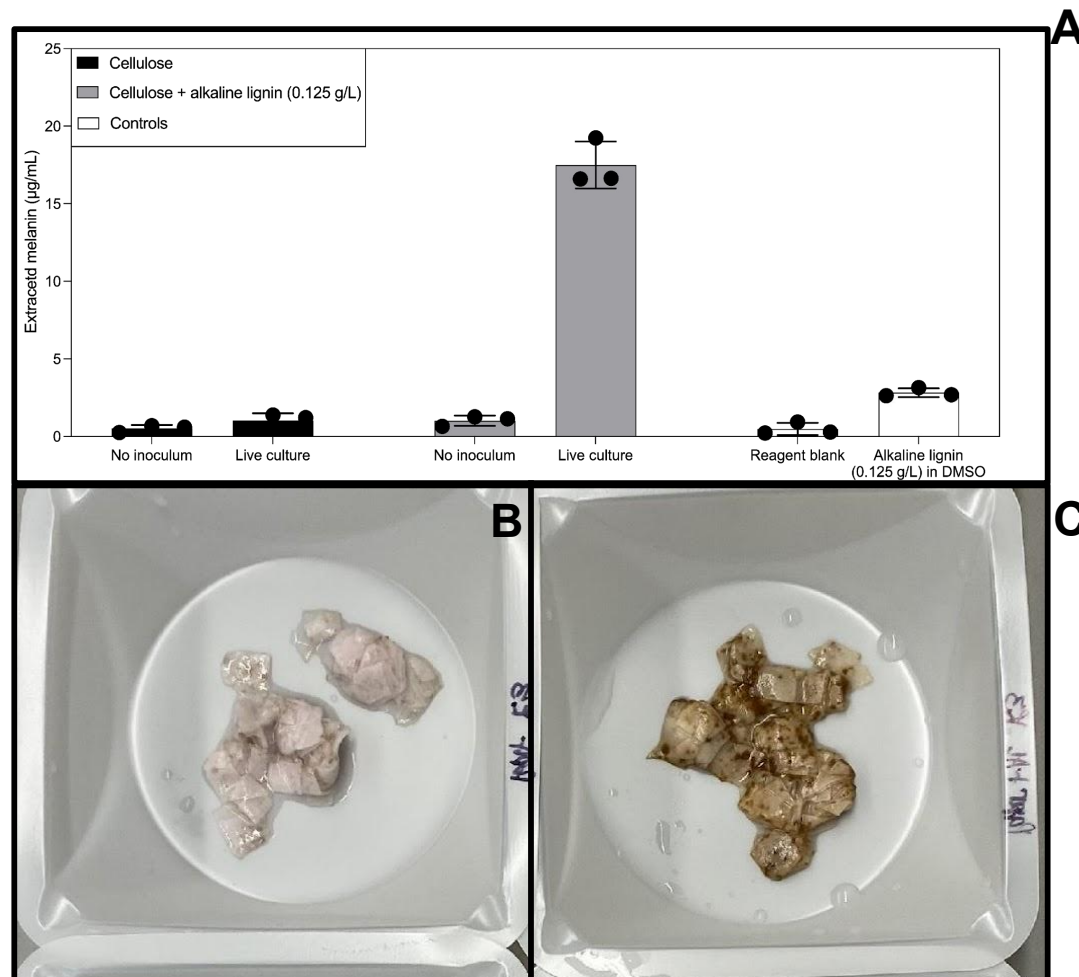


Figure caption: A) Biochemical evidence of melanin production by AGF by introduction of lignin. B) Fungi without melanin (grey/tan) C) Fungi with melanin (brown).

Lankiewicz, T. S., et al. Biotechnology for Biofuels and Bioproducts. doi: 10.1186/s13068-025-02696-5 (JBEI #1261)

A large-scale screening campaign of putative carbohydrate-active enzymes reveals a novel xylanase from anaerobic gut fungi

Background/Objective

- Efficient breakdown of plant biomass is crucial to produce high-value bio-based products, but identifying enzymes that reduce deconstruction costs remains a significant challenge
- Anaerobic gut fungi (AGF) are attractive alternative sources for enzymes due to their specialization in plant biomass deconstruction

Approach

- Here we developed a bioinformatic pipeline to annotate novel putative CAZymes from anaerobic fungi, specifically *Piromyces finnis*, followed by heterologous expression in *E. coli*
- This study leveraged cutting-edge computational tools such as AlphaFold and FoldSeek for *in silico* protein structure and function prediction to accelerate the screening process

Results

- Out of 173 fungal CAZymes expressed in *E. coli*, only 9.8% were soluble with expression levels exceeding 5% of the total proteome, resulting in a subset of 17 well-expressed and soluble protein candidates for further analysis
- Ten of the 17 selected proteins demonstrated activity against one or more substrates including the identification of a novel xylanase, celsome_012 which produced five times more product than the second-most active xylanase screened

Significance/Impacts

- This work represents a promising step toward the industrial translation of anaerobic fungal CAZymes for lignocellulose valorization, highlighting their robust lignocellulolytic activity and providing a framework for prioritizing AGF proteins for efficient *E. coli* heterologous expression

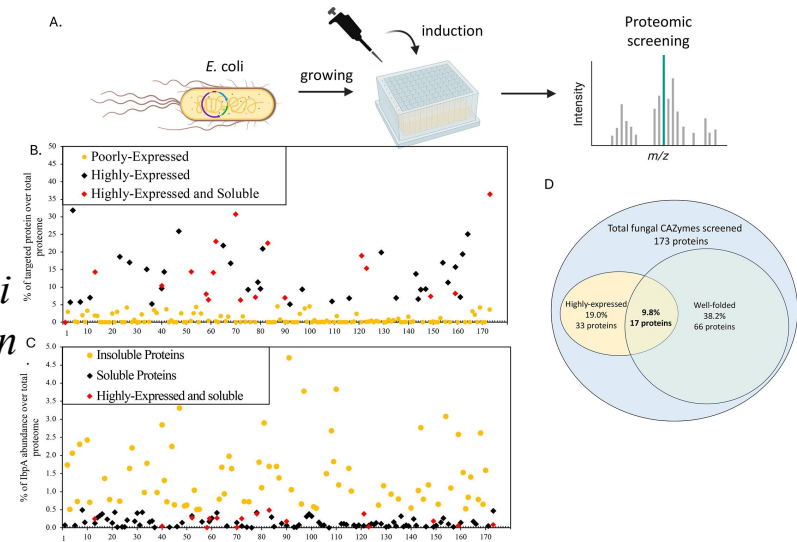


Figure: (A) Workflow for proteomic screening of 173 CAZymes identified from AGF strain *P. finnis*. (B) Distribution of fungal CAZyme expression levels in *E. coli*. (C) Distribution of IbpA production during fungal protein expression in *E. coli* was used as an indicator of protein folding success and solubility. (D) Seventeen highly and soluble proteins from AGF were further characterized in *E. coli*.

Comparison of the efficacy of a biocompatible and a distillable solvent for pretreatment of mixed bioenergy feedstocks

Background/Objective

- Identifying and selecting an optimal pretreatment solvent that enhances enzymatic saccharification while being cost-efficient and ensuring sustainability remains a challenge.
- To Compare the effectiveness of the biocompatible ionic liquid against the distillable solvent for the pretreatment of mixed bioenergy feedstocks

Approach

- Used two solvents and three biomass for pretreatment, and saccharification
- Studied solvent removal and biocompatibility to reveal process trade off

Results

- Ethanolamine enhances glucose yield ~22% to 52% compared to [Ch][Lys]
- The highest sugar release observed for the combination of poplar, switchgrass and sorghum with ethanolamine, achieving a glucose yield of ~85% and a xylose yield of ~80%.

Significance/Impacts

- This study contributes to the development of economically viable and scalable pretreatment technologies for bioenergy applications using different feedstocks and process configurations.

Rahman, M. M., et al. *Biomass and Bioenergy*. doi: 10.1016/j.biombioe.2025.108333 (JBEI #1263)

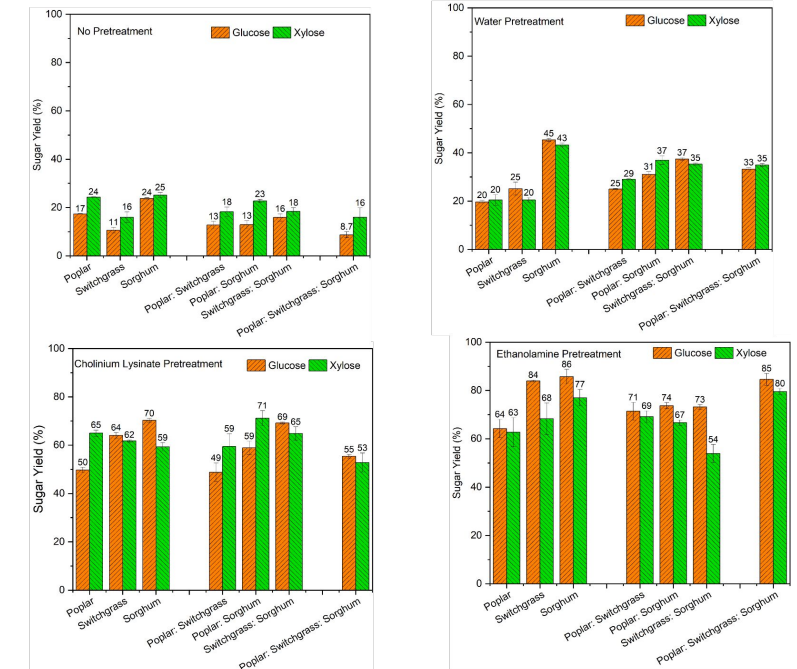
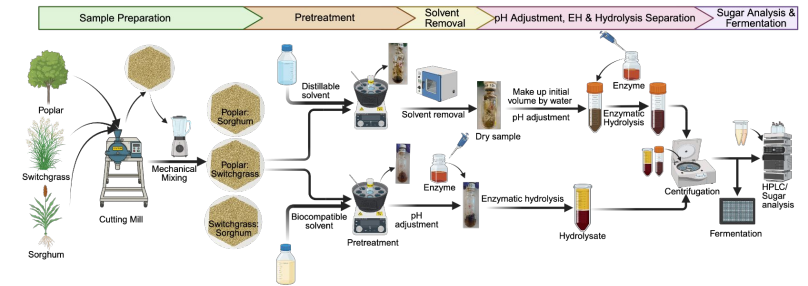


Figure caption: Process flow for deconstruction of mixed feedstocks with a biocompatible and a distillable solvent (top); glucose and xylose yield from untreated and water pretreated biomass (middle); glucose and xylose yield from cholinium lysinate and ethanolamine pretreated biomass.

Enabled Publications

Life-cycle carbon footprint and total production potential of cross-laminated timber from California's wildland-urban interface

Background/Objective

- Forest residues are often pile or broadcast burned as part of forest management operations. Understanding how much and where they are is important for converting them to bioenergy and bioproducts.

Approach

- We modeled forest management activities using scenarios from forestry experts at Yale, assembled values for accessible areas and estimated CLT production potential and emissions impacts.

Results

- Forest residue availability is enormous in CA and in other regions where wildfire risk is high. But downselecting to areas likely to undergo management, and where wood is accessible is key.

Significance/Impacts

- While this study focused on CLT production, forest residues have potential as a bioenergy feedstock. These estimates can broaden the analysis of feedstock availability for fuel and chemical production.

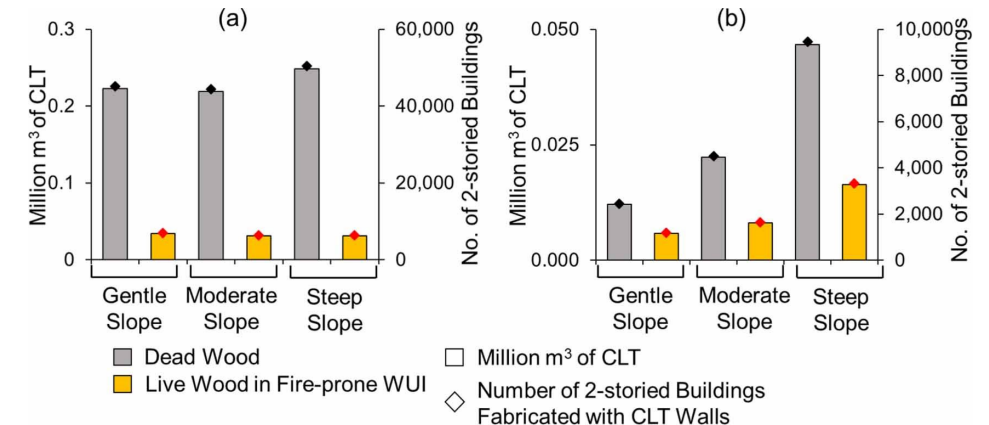
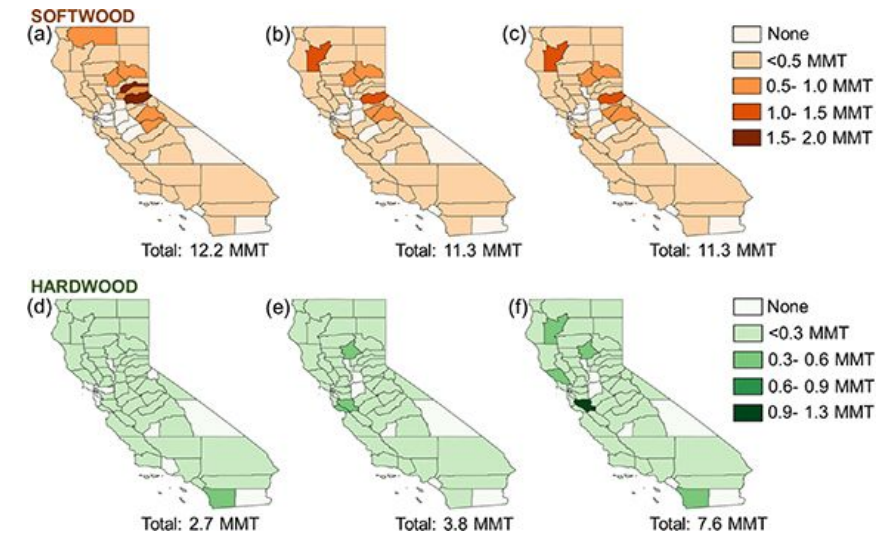


Figure caption: Forest residue availability suitable for CLT (top), Forest residue and CLT production potential depending on slope in CA (bottom)